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Alterations of visual evoked potentials in preschool Inuit children exposed to methylmercury and polychlorinated biphenyls from a marine diet☆

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Abstract

The aim of the present study was to assess the impact of chronic exposure to polychlorinated biphenyls (PCBs) and methylmercury on visual brain processing in Inuit children from Nunavik (Northern Québec, Canada). Concentrations of total mercury in blood and PCB 153 in plasma had been measured at birth and they were again measured at the time of testing in 102 preschool aged children. Relationships between contaminants and pattern-reversal visual evoked potentials (VEPs) were assessed by multivariate regression analyses, taking into account several potential confounding variables. The possible protective effects of selenium and omega-3 polyunsaturated fatty acids against methylmercury and PCB toxicity were also investigated. Results indicate that exposure to methylmercury and PCBs resulting from fish and sea mammal consumption were associated with alterations of VEP responses, especially for the latency of the N75 and of the P100 components. In contrast, the concomitant intake of omega-3 polyunsaturated fatty acids was associated with a shorter latency of the P100. However, no significant interactions between nutrients and contaminants were found, contradicting the notion that these nutrients could afford protection against environmental neurotoxicants. Interestingly, significant associations were found with concentrations of neurotoxicants in blood samples collected at the time of testing, i.e. at the preschool age. Our findings suggest that VEP can be used as a valuable tool to assess the developmental neurotoxicity of environmental contaminants in fish-eating populations.
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1. Introduction

The toxicity of methylmercury and polychlorinated biphenyls (PCBs), two of the most prevalent and ubiquitous environmental contaminants, was first recognized decades ago following accidental exposures. Reports from Japan in the 1950s and Iraq in the 1970s showed that prenatal exposure to very high doses of methylmercury could lead to mental retardation, motor damages, ataxia and seizures (Harada, 1995; Marsh et al., 1977). Moreover, it was observed in the late 1960s and 1970s that acute exposure to PCBs, in Japanese and Taiwanese infants born to women highly exposed to PCBs – also containing polychlorinated dibenzofurans – led to skin

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HCB, hexachlorobenzene; PCBs, polychlorinated biphenyls; *n*-3 PUFAs, omega-3 polyunsaturated fatty acids; VEPs, visual evoked potentials; ERPs, event-evoked potentials

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rashes and intellectual deficits during infancy and childhood (e.g. Chen et al., 1992).

More recently, three major prospective cohort studies have examined the relation between neurotoxicity and exposure to mercury through seafood consumption in coastal populations. Since most of the mercury in the marine food chain is methylmercury, total blood mercury concentrations measured in these studies reflect exposure to the neurotoxic form of mercury. Impairments in attention, memory, intellectual performance, balance and motor abilities were associated with blood mercury levels in the Faroe Islands (Grandjean et al., 1997) and New Zealand (Crump et al., 1998). These deficits, however, were not observed in a similar study conducted in the Seychelles Islands (Myers et al., 1995a).

In addition to the observed cognitive impairments associated with blood mercury concentrations, it has been reported that blood mercury concentrations were related to alterations of sensory function, especially vision. In adults monkeys and humans, methylmercury exposure has been linked to constriction of the visual field and abnormal color vision (Chang and Verity, 1995; Korogi et al., 1997; Lebel et al., 1996; Merigan et al., 1983). Although a lack of association between prenatal methylmercury exposure and contrast sensitivity was reported in Faroese children (Grandjean et al., 1997, 2001b), other studies conducted in young animals (Rice and Gilbert, 1982, 1990) and children (Altmann et al., 1998; Hudnell et al., 1996) have shown impairments in contrast sensitivity following long-term prenatal and perinatal exposure to methylmercury.

In cohort studies conducted in fish-eating and in general populations, prenatal exposure to PCBs has been associated with impaired psychomotor development (Gladen et al., 1988; Rogan and Gladen, 1991), intellectual function (Jacobson and Jacobson, 1996; Jacobson et al., 1990; Patandin et al., 1999), visual memory (Darvill et al., 2000; Jacobson et al., 1990) and attention (Jacobson and Jacobson, 2003). The potential effects of PCB exposure on visual functions, however, have seldom been examined. To our knowledge, only Kilburn (2000), testing an adult population, reported color discrimination impairments and visual field constriction in relation to postnatal PCB exposure.

Further insights about the integrity of the visual system in children exposed to these environmental contaminants could be obtained from the scalp-recording of visual evoked potentials (VEPs), a electrophysiological technique commonly used in pediatric populations. Since VEPs reflect the maturation and the functional integrity of the visual system, damage along visual pathways leads to abnormal VEP latency and/or amplitude. Pattern reversal stimulation (checkerboards or sinusoidal gratings) typically evokes a triphasic wave with components traditionally labeled according to polarity (positive or negative peak) and peak latency in millisecond, i.e. N75, P100 and N145 or N150 (Halliday, 1992; Odom et al., 2004). VEPs – by contrast to event-related potentials (ERPs) such as the visual P300 – are ideal to evaluate the integrity of the so-called exogenous components, i.e. the early components, directly modulated by the physical attributes of the stimulus, that occur less than 200 ms after stimulus onset. VEPs could therefore be very effective in assessing whether the initial brain processing of visual information is impaired by chronic exposure to environmental contaminants.

Prenatal methylmercury exposure has been associated with a significant delay of the N145 component of the VEP in Portuguese preschool children (Murata et al., 1999a,b), but this result was not corroborated in the Faroese cohort (Grandjean et al., 1997; Murata et al., 1999a,b; Weihe et al., 2002). Two reasons may explain this inconstancy. First, only standard basic visual stimulation, i.e. relatively large checkerboards (30 and 15 arc min) and high contrast, were presented in these studies. Such supra-threshold stimuli might introduce ceiling effects and reduce the likelihood of observing significant outcomes. Second, these studies have not considered the putative protection against mercury-induced toxicity that could be afforded by nutrients such as the omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) and selenium. Indeed, it is known that *n*-3 PUFAs supplements during the first months of life are associated with faster maturation of the visual system and better visual acuity in infants, and there is evidence from animal studies that selenium could influence the disposition of mercury in the body and offer protection against its toxicity (National Research Council, 2000). The importance of controlling for such confounds is also true for PCB toxicity. To our knowledge, no study has reported the adverse effects of PCB exposure on early VEP components in children, although prenatal PCB exposure has been related to longer latencies and reduced amplitudes of the visual P300 component (Chen and Hsu, 1994; Vreugdenhil et al., 2004). This apparent absence of a correlation between VEP alteration and PCB exposure in fish-eating populations might be due to a protective effect of *n*-3 PUFAs and/or selenium that are also found in seafood.

The present study was designed to assess the neurotoxicity associated with pre- and postnatal exposure to methylmercury and PCBs, using VEPs in preschool Inuit children living in Nunavik (Northern Québec, Canada) where total mercury and PCB concentrations measured in Inuit newborns are much higher than those observed in the general population in North America (Muckle et al., 2001b). Since fish and marine mammals represent an important part of their diet, exposure of the Inuit population to methylmercury is in the same range as those reported in the major studies conducted in fish-eating populations (Dewailly et al., 1996; Muckle et al., 2001a,b). As for PCB exposure in the Inuit population, it is similar to that found in studies conducted in the Netherlands (Vreugdenhil et al., 2002). In order to increase the sensitivity of VEPs to subtle neurological dysfunctions associated with exposure to mercury and PCBs, we used high spatial resolution (spatial frequency) stimuli and three levels of contrast (high, medium and low). In addition to this stimulus saliency manipulation, we aimed to maximize our protocol by controlling several confounds. Hence the putative protective effect of *n*-3 PUFAs and selenium on methylmercury- and PCB-induced neurotoxicity was assessed. We hypothesize that methylmercury and PCB alter VEP responses differently, according to the current status of selenium and *n*-3 PUFAs, respectively. Elevated intake of these nutrients could eliminate or attenuate the neurotoxic effects of exposure to these environmental contaminants. In contrast to the studies that have

investigated the effects of only one contaminant, our Inuit cohort was exposed to several contaminants and dietary factors simultaneously through fish consumption. Therefore, the assessment of the impact of methylmercury and/or PCB exposure in fish-eating populations required the consideration of several confounds simultaneously (including moderators and other environmental contaminants) as well as the potential interactions that can occur between all these variables.

2. Methods

2.1. Participants

Among the 483 newborns who had participated in the Nunavik Cord Blood Monitoring Program, in which several persistent organic pollutants had been measured in umbilical cord blood (Muckle et al., 1998), 110 preschool children, aged 5 to 6 years (mean = 5.4 ± 0.4), have been successfully recruited. Detailed information on eligibility, inclusion criteria and participation rate for this sample have been presented elsewhere (Despres et al., 2005). In order to document a broad range of potential confounding variables, a detailed interview was conducted with the mothers to gather socio-demographic information and evaluate the quality of the stimulation provided to the child in the family setting. The research procedures were approved by Sainte-Justine Hospital and Laval University ethics committees, and an informed consent was obtained from a parent of each participant.

2.2. Visual evoked potentials

Vertical reversal sinusoidal gratings having a spatial frequency of three cycles per degree were generated with Pixx™ software and were displayed on a ViewSonic P815 monitor (1024H × 728V, 120 Hz). Stimuli were presented for 1 s with a reversal rate of 1 Hz at three different contrast levels: high-level (95%), mid-level (30%) and low-level (12%). Subjects viewed the stimuli (24° × 24°) binocularly, from a distance of 1 m in a dimly lit room. They were instructed to fixate a small red dot located in the center of the screen. The electrophysiological recordings were interrupted if the reflection of the stimulus was not centered over the pupil, as controlled by an observer. Data were recorded with an INSTEP system. The electro-oculogram (EOG) was recorded from the outer canthus of each eye (horizontal EOG) and above and below the right eye (vertical EOG). Pattern-reversal VEPs were recorded from Oz derivation according to the international 10–20 system from an Ag–AgCl electrode. The reference and the ground electrodes were located on the nose and the forehead, respectively. Impedance was kept below 5 kΩ. The EEG signal was amplified and band-pass filtered at 0.1–100 Hz. Between 50 and 60 trials were recorded in each condition, namely at contrast 95%, 30% and 12%. The pattern-reversal VEPs waves were time-locked to the stimulus and averaged (sweep time, 500 ms; pre-stimulus delay, 50 ms; sampling rate, 1000 Hz). Trials in which the response was higher than 75 μV at any recording site (horizontal EOG, vertical EOG or Oz) were rejected before averaging in order to eliminate ocular and muscular artefacts. The following standard VEP components (Odom et al., 2004) were examined: N75 (negative deflection at ≈75 ms), P100 (positive deflection at ≈100 ms) and N150 (negative deflection at ≈150 ms). For each component, the latency was determined from the stimulus onset to the maximal waveform peak, whereas the amplitude was calculated from peak-to-peak procedure (N75-to-P100 and P100-to-N150) (Fig. 1). The determination of the latency and of the amplitude for the different components was performed by two independent electrophysiologists blind to chemical exposures. When there was a discrepancy in the amplitude and/or latency determination between the two raters, the average of the two measures was taken; inter-rater agreement was high ($r = 0.98$).

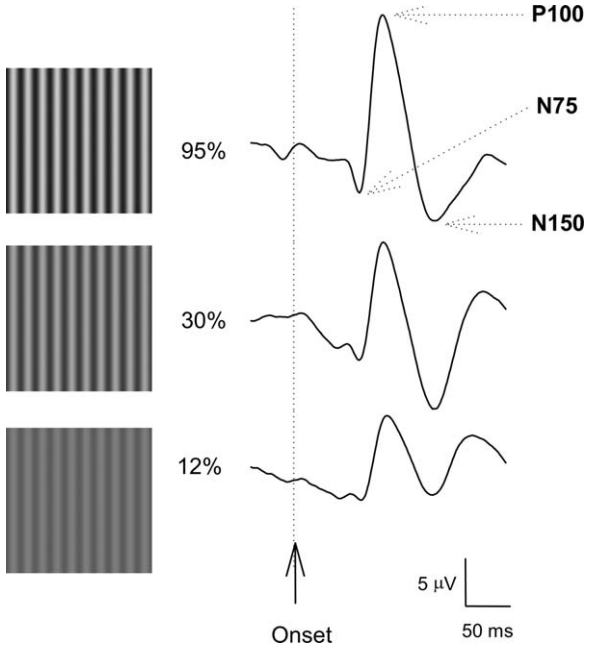

Fig. 1. VEP grand mean average recorded at Oz. Pattern-reversal VEPs typically show three major components: N75, P100 and N150. Latencies and peak-to-peak amplitudes were measured for each VEP components at three contrast levels: 95% ($n = 78$), 30% ($n = 75$) and 12% ($n = 66$).

2.3. Biological measures and laboratory procedures

Blood samples collected at birth from the umbilical cord and at testing time from the participating children were used to determine concentrations of PCBs, chlorinated pesticides, total mercury, selenium, *n*-3 PUFAs and lead at the time of testing. A hair sample (5-mm diameter and 1 cm length) was also collected at the time of testing and analyzed for total mercury. The analyses were performed at the *Laboratoire de Toxicologie INSPQ*, which is accredited by the Canadian Association for Environmental Analytical Laboratories. Detailed analytical and quality control procedures were described previously (Muckle et al., 2001a; Rhainds et al., 1999). Briefly, the 14 most prevalent PCB congeners (IUPAC nos. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180,

183, 187) and 11 chlorinated pesticides or their metabolites (aldrin, α-chlordane, γ-chlordane, *pp′*-DDT, *pp′*-DDE, HCB, β-HCH, mirex, *cis*-nonachlor, *trans*-nonachlor, oxychlordane) were measured in purified plasma extracts using high-resolution gas chromatography (Hewlett-Packard HP5890A), with two capillary columns (Hewlett-Packard Ultra I and Ultra II) and dual Ni-63 electron capture detectors. Total mercury concentrations were determined in blood and hair samples using cold vapor atomic absorption spectrometry (Pharmacia Model 120). Blood lead concentrations were measured by graphite furnace atomic absorption with Zeeman background correction (Perkin-Elmer model ZL4100) and blood selenium levels were assessed by inductively coupled plasma-mass spectrometry (PE Elan 6000; Perkin-Elmer). The fatty acid profile in total plasma phospholipids was determined by capillary gas–liquid chromatography, after transmethylation of the fatty acids. In all analyses, whenever a ''not detected'' result was obtained, a value equal to half the limit of detection of the analytical method was entered in the database. The detection limits were 1.0 nmol/L for blood mercury, 1.0 nmol/g for hair mercury, 50 nmol/L for blood lead, 0.1 μmol/L for blood selenium and 0.02 μg/L for all PCB congeners and chlorinated pesticides in plasma.

2.4. Statistical analyses

A broad range of potential confounding variables was documented from maternal interviews and blood analysis. They were selected for their potential or documented associations with the dependent variables (Table 1). PCB congener 153 was used as the marker for exposure to organochlorine mixture because it is highly correlated with other PCB congeners and chlorinated pesticides, and is considered a good marker of exposure to environmental PCB mixture in the Arctic (Muckle et al., 2001a; Ulbrich and Stahlmann, 2004). As described in detail elsewhere (Despres et al., 2005), PCB congener 153 was the most prevalent congener, representing 31.3% and 34.3% of total PCB mixture in cord and child plasma samples, respectively, in the initial sample ($n = 110$). Furthermore, PCB 153 was highly correlated to all other PCB congeners: correlations ranged from 0.84 to 0.98 for cord samples and from 0.91 to 0.99 for child plasma samples. Statistical analyses were performed using total mercury concentrations in child blood to document current methylmercury exposure since child blood and hair mercury concentrations were highly correlated ($r = 0.91$). Cord blood selenium concentrations were not included in the

Table 1
Descriptive statistics of potential confounding variables

	n	Mean	S.D.	Range
Child characteristics				
Age at testing	78	5.4	0.4	4.8–6.1
Sex (% females)	78	61.5		
Breastfeeding duration (week)[a]	77	59.1	74.3	0.0–258.0
Weight at birth (kg)	77	3.5	0.5	2.6–4.6
Weight at testing (kg)	77	21.5	3.5	16.3–44.4
Head size at birth (cm)	72	35.0	2.2	31.0–50.0
Head size at testing (cm)	76	51.5	3.9	19.6–54.6
Height at birth (cm)	73	50.9	2.0	46.5–56.0
Height at testing (cm)	78	110.0	4.3	101.3–121.6
Child hemoglobin at testing (g/L)	78	123.3	13.0	88.0–172.0
Maternal and family characteristics				
Parity	78	4.2	1.8	1.0–8.0
Maternal socio-economic status (SES)[b]	76	29.1	11.7	8.0–53.5
Highest grade completed by caregiver at testing (years)[c]	73	9.2	2.4	0.0–16.0
Number of children and adults at home at testing	78	6.5	2.3	2.0–13.0
Psychological distress of primary caregiver at testing[d]	68	23.2	5.4	14.0–36.0
Maternal non verbal reasoning abilities[e]	73	35.7	7.8	19.0–51.0
Intra-family violence for the year prior to testing[f]	38	65.7	66.1	0.0–240.0
Other prenatal exposures				
Cord blood lead (μmol/L)	78	0.3	0.2	0.1–1.3
Child blood lead (μmol/L)	78	0.2	0.2	0.1–1.8
Binge drinking during pregnancy (% ≥ 5 standard drinks of alcohol per occasion)	74	17.6		
Marijuana use during pregnancy (% yes)	74	18.9		
Smoking during pregnancy (% > 10 cigarettes/day)[g]	76	36.8		

S.D. = standard deviation.
[a] 78.2% were breastfed.
[b] Hollingshead index for the mother and her partner or, if she was not self-supporting, for her primary source of support (Hollingshead, 1975).
[c] 96.2% were raised by their biological mother, two children were adopted, one child was in foster care.
[d] IDESQ (Préville et al., 1992).
[e] Raven Progressive Matrices (Raven et al., 1992).
[f] Conflict Tactics Scale (Strauss, 1979).
[g] 87.9% smokers during pregnancy.

analyses because data were missing for 38 participants. PCB 153, total mercury and selenium concentrations followed log-normal distributions and analyses were therefore conducted with natural log-transformed values. Based on the fact that docosahexaenoic acid (DHA) in retina and central nervous system development is predominant in the perinatal period (Neuringer and Jeffrey, 2003) and that eicosapentaenoic acid (EPA) is better associated with fish consumption (Silverman et al., 1990), DHA and EPA were considered as *n*-3 PUFA markers for umbilical cord blood and child blood, respectively.

Pearson correlation analyses were performed to select, among the potential confounding variables listed in Table 1, those to be included in subsequent analyses. Any variable associated with a specific outcome at p-value ≤ 0.20 was included as a potential confounding variable in a multiple regression analysis with this outcome. To investigate the associations between prenatal exposure to environmental contaminants and the dependent variables (VEP latencies of N75, P100, N150 and peak-to-peak amplitudes of N75-to-P100 and P100-to-N150), the following were simultaneously included in multiple regressions: the independent variables cord PCB 153 and cord mercury, cord DHA, the potential confounders as well as the cord PCB 153/cord DHA interaction. Final regression models for prenatal exposure were obtained for each outcome by removing, one at a time, the potential confounding variables and interactions that were not significantly associated with the outcome ($p \geq 0.10$) and the other variables in the regression.

To investigate the effect of postnatal exposure, the strategy described above was retained with child PCB 153 and child mercury as independent variables, child EPA and child selenium as protective variables, and child PCB 153/child EPA as well as child mercury/child selenium as interaction factors. The prenatal variables found to be significant in previous models were also included in this analysis. Outcome variables were normally distributed, as well as the residuals of the retained regression models, and the absence of multicollinearity was tested and confirmed. All statistical analyses were performed using the SAS v8.2 software (SAS Institute, Inc., Cary, NC).

3. Results

Electrophysiological data was gathered for 102 children (56% females) from different communities along the Hudson Coast (48%) and Ungava Coast (52%). The average age was 5.44 years (range from 5.07 to 5.81 years). Adequate electrophysiological data were obtained for 78 of the 102 tested children. Inadequate data were due to technical/computer problems ($n = 1$), lack of visual screening and collaboration ($n = 3$), insufficient signal to noise ratio ($n = 11$) and abnormal visual acuity (Snellen E chart) in one or both eyes, i.e. $\geq$20/40 ($n = 9$). Vision was considered normal when visual acuity ranged from 20/20 to 20/30, taking into consideration that testing conditions were not as optimal as in a clinical setting.

3.1. Descriptive statistics

As illustrated in Table 2, the mean VEP amplitude decreased and the latency increased as a function of contrast, especially for the N75 and P100 components. The corresponding waveforms are plotted in Fig. 1. At low contrasts, the signal-to-noise ratio was too low for some participants ($n = 3$ at 30% contrast and $n = 12$ at 12% contrast) to reliably quantify the waveforms and these participants were excluded from the analyses. Such VEP modulation as a function of contrast is typically observed in the literature (e.g. Roy et al., 1995).

Descriptive statistics for contaminants and nutrients are presented in Table 3. Concentrations of contaminants and nutrients collected at birth or at time of testing for the 78 children successfully tested did not differ from the original sample of 102 (data not shown).

3.2. Intercorrelations between contaminants and nutrients

The intercorrelations between PCB 153, total mercury, selenium and *n*-3 PUFA concentrations measured in cord and child blood are presented in Table 4. Cord mercury and child mercury concentrations are moderately associated, as are cord PCB 153 with child PCB 153 concentrations. Moreover, child PCB 153 concentrations are well predicted

Table 2
Descriptive statistics of VEPs obtained at 95%, 30% and 12% of contrast levels

Contrast	*n*	Mean	S.D.	I.Q.R.
Latencies (ms)				
95%				
N75	78	75.4	8.1	73.0–81.0
P100		107.0	7.9	103.0–110.0
N150		160.1	17.8	148.0–169.0
30%				
N75	75	76.7	8.0	73.0–82.0
P100		106.9	10.5	100.0–112.0
N150		158.7	14.1	151.0–168.0
12%				
N75	66	81.1	9.5	77.0–85.0
P100		116.8	16.8	107.0–121.0
N150		166.9	23.6	155.0–177.5
Amplitudes (μV)				
95%				
N75–P100	78	35.5	17.1	22.6–46.3
P100–N150		34.7	18.6	18.2–45.1
30%				
N75–P100	75	22.5	10.9	13.2–30.6
P100–N150		29.2	14.7	19.4–38.0
12%				
N75–P100	66	19.3	8.3	12.8–24.2
P100–N150		21.1	10.3	13.5–28.9

For each component (N75, P100 and N150), the latency was determined from the stimulus onset to the maximal waveform peak, whereas the amplitude was calculated from peak-to-peak (N75-to-P100 and P100-to-N150). S.D. = standard deviation, I.Q.R. = interquartile range.

Table 3
Descriptive statistics of the environmental contaminants and nutrients concentrations measured in cord and child blood samples

	n	Geometric mean (95% CI)	Arithmetic mean	S.D.	Range
Contaminants					
Cord mercury (nmol/L)	78	82.40 (67.00–101.50)	119.30	101.50	9.00–520.00
Child mercury (nmol/L)	78	29.50 (22.70–38.40)	49.30	45.50	1.00–191.00
Cord PCB 153 (μg/kg of lipids)	77	98.02 (85.76–112.04)	115.96	70.23	23.09–387.05
Child PCB 153 (μg/kg of lipids)	77	83.17 (63.85–108.32)	152.45	175.30	7.46–777.80
Nutrients					
Cord selenium (μmol/L)	39	4.04 (3.52–4.64)	4.44	2.08	2.07–9.80
Child selenium (μmol/L)	78	4.19 (3.64–4.84)	5.43	5.40	2.00–32.50
Cord DHA (% phospholipids)	71	3.17 (2.91–3.45)	3.36	1.09	1.12–6.22
Child EPA (% phospholipids)	77	0.33 (0.28–0.40)	0.48	0.48	0.06–2.52

DHA and EPA concentrations are expressed in percentage according to plasma phospholipids. PCB = polychlorinated biphenyl congener IUPAC 153, DHA = docosahexaenoic acid (22:6 *n*-3), EPA = eicosapentaenoic acid (20:5 *n*-3), S.D. = standard deviation, and I.Q.R. = interquartile range.

from cord PCB and breastfeeding duration, as revealed by regression analysis: cord PCB (standardized $\beta = 0.38$, $p < 0.0001$) and breastfeeding duration (standardized $\beta = 0.65$, $p < 0.0001$) accounted for 56% of the total variance of blood PCB 153 concentration at the time of testing. As expected, the correlations between mercury and PCB 153 are in the moderate range, and are stronger in the umbilical cord blood than in child blood samples. Significant positive associations are observed between child selenium and child mercury concentrations, cord DHA and cord mercury, child EPA and child selenium, and these associations are in the low to moderate range.

3.3. Multivariate regression analyses

Since the probability plots showed evidence that the outcomes were normally distributed, as were the residuals from the regression models, the ordinary least squares method could be used to investigate associations between VEPs and concentrations of contaminants or nutrients. Because of the ''stepwise'' approach used in the regression analyses (see Section 2), only the final variables (after adjustment for covariables) are shown in Table 5. Since none of the interaction factors between the exposure variables and the nutrients of interest (cord PCB/cord DHA, child PCB/child EPA, child mercury/child selenium) were significantly related to VEP latencies and amplitudes, further regressions analyses only included blood mercury concentration at birth and at the time of testing as well as and PCB 153 at the time of testing. The finals models retained do not all include the same potential protective factors and the same confounding variables. After controlling for confounders, blood mercury concentrations at time of testing were associated with shorter latencies of the early N75 component at 95% and 30% contrasts, the P100 component at 95% contrast ($p \leq 0.001$), and the P100 at 30% contrast ($p \leq 0.01$). The β coefficients indicate that an increase of mercury concentration of one unit of natural logarithm (i.e. by a factor of ~2.7) is associated with a decrease in latency in the order of 3–4 ms. Cord mercury concentrations were associated with longer latencies of the P100 component at 30% contrast. Blood selenium concentrations at testing time were related to longer N75 latencies at 95% ($p \leq 0.001$) and 30% ($p \leq 0.01$) contrasts as

Table 4
Intercorrelations between PCB 153, mercury, selenium and *n*-3 fatty acids (DHA and EPA) concentrations sampled from cord blood and child blood

	Mercury (log)		PCB 153 (log)		Selenium (log)		DHA cord	EPA child
	Cord	Child	Cord	Child	Cord	Child		
Mercury (log)								
Cord	1	0.45*** (78)	0.47*** (77)	0.35** (77)	0.26 (39)	0.14 (78)	0.27* (71)	−0.08 (77)
Child		1	0.28* (77)	0.32** (77)	0.25 (39)	0.55*** (78)	0.13 (71)	0.17 (77)
PCB 153 (log)								
Cord			1	0.38*** (76)	0.15 (39)	0.18 (77)	0.10 (71)	0.05 (76)
Child				1	0.17 (38)	0.17 (77)	0.27*(70)	0.11 (77)
Selenium (log)								
Cord					1	0.40* (39)	0.26 (35)	0.05 (38)
Child						1	0.13 (71)	0.25*(77)
DHA cord							1	0.09 (70)
EPA child								1

PCB 153 = polychlorinated biphenyl congener IUPAC 153, DHA = docosahexaenoic acid (22:6 *n*-3), and EPA = eicosapentaenoic acid (20:5 *n*-3).
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.002$.

Table 5
Regression coefficients of PCB 153, mercury and eicosapentaenoic acid (EPA) after adjustment for confounding variables

Contrast (%)	Variables	VEP latency					
		N75		P100		N150	
		β	R^2	β	R^2	β	R^2
95	Child mercury	-3.90^{***}	0.30	-3.26^{***}	0.39	1.35	0.16
	Child PCB 153	0.10		2.50^{**}		0.57	
	Child selenium	6.40^{***}		4.83^{**}		##	
	Child EPA	##		-5.71^{**}		##	
	n	72		70		71	
30	Child mercury	-3.18^{***}	0.25	-3.94^{**}	0.29	−1.15	0.21
	Cord mercury	##		3.34^{*}			
	Child PCB 153	0.54		1.12		1.42	
	Child selenium	5.48^{**}		5.79^{**}		5.24^{+}	
	Child EPA	##		##		-7.82^{*}	
	n	69		71		71	
12	Child mercury	−0.93	0.14	−0.47	0.15	1.85	0.20
	Child PCB 153	−0.44		3.22^{+}		5.58^{*}	
	n	62		61		61	

Contrast (%)	Variables	VEP amplitue			
		N75-to-P100		P100-to-N150	
		β	R^2	β	R^2
95	Child mercury	−0.57	0.12	1.69	0.22
	Child PCB 153	-3.74^{*}		-3.17^{+}	
	Child selenium	##		##	
	Child EPA	##		##	
	n	76		72	
30	Child mercury	−0.90	0.11	0.93	0.20
	Child PCB 153	−1.24		−1.45	
	Child selenium	##		##	
	Child EPA	##		##	
	n	73		71	
12	Child mercury	0.58	0.11	−0.15	0.31
	Child PCB 153	0.15		0.98	
	Child selenium	##		##	
	Child EPA	##		-5.97^{*}	
	n	64		64	

Covariables included in the models were alcohol (N75-95%, P100-95%, 30% and 12%, N150-30% and 12%), marijuana (P100-95%, N75-12%, P100-to-N150 at 95% and 30%), maternal non-verbal reasoning abilities (N150-95%), hemoglobin concentrations at testing time (N150-95% and 30%), highest grade completed by the primary caregiver (N75-30%), number of children and adults at home (P100-95% and 30%), sex (N150-12%, N75-to-P100 at 95%, P100-to-N150 at 95% and 12%), parity (N75-to-P100 at 30% and 12%, P100-to-N150 at 95%, 30% and 12%) and height at birth (P100-to-N150 at 12%).

$^{+}$ $p \leq 0.10$.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.002$.
Excluded variables in the final model because of the absence of significance and confounding effects.

well as with longer P100 latency at 95% and 30% contrasts ($p \leq 0.01$). A tendency for longer latency for the N150 components at 30% contrast was observed with increasing selenium concentrations ($p \leq 0.10$).

Plasma PCB 153 at testing time was significantly related to longer P100 latency at 95% and 30% contrast and N150 latency at 12% contrast. A tendency for such associations was also observed for the P100 at 12% contrast. EPA was significantly related to shorter VEP latencies of the P100 at 95% contrast and of the N150 at 30% contrast. The regression models on latencies were all significant ($p \leq 0.05$) and the models accounted for 14 to 39% of the variance.

The amplitude of the N75-to-P100 at 95% contrast was significantly reduced as a function of increased child PCB 153 concentrations, and this significant model explained 12% of the total variance. A tendency towards a reduction of the amplitude of the P100-to-N150 at 95% contrast with increased child PCB 153 concentrations was also noted ($p \leq 0.10$). These associations are in agreement with those observed between latency and PCB 153, indicating that PCB

153 alters both latency and amplitude of VEPs. Since several chlorinated pesticides detected in the cord and child plasma samples were very highly correlated with PCB 153 (correlation coefficients from 0.77 to 0.88 in cord plasma and from 0.91 to 0.96 in child plasma), an additional regression was conducted to examine whether the associations observed with PCB 153 could also be found with chlorinated pesticides. To this end, the chlorinated pesticide that was the least correlated to PCB 153 (r with PCB 153: cord = 0.77, child = 0.91), namely the hexachlorobenzene (HCB), was selected. Similar results were obtained when HCB was included in regression analyses instead of PCB 153, both with VEP latencies or amplitudes. On the other hand, the amplitude of the P100-to-N150 at 12% contrast was significantly reduced as a function of increased child EPA concentrations, and this significant model explained 31% of the total variance.

4. Discussion

The present study investigated the latency and the amplitude of VEPs to assess the impact of exposure to environmental contaminants on visual processing. To this end, a VEP protocol was designed to optimally detect sub-clinical effects, and the research design was developed to take into account mercury and PCBs as well as the potential protective effects of nutrients that co-occur with exposure to these contaminants through fish and sea mammal consumption. Independently of the effects of nutrients, blood concentrations of mercury and PCBs – especially those measured at the time of testing – were found to be clearly associated with sub-clinical effects on the visual system.

Grandjean and coworkers reported that prenatal methylmercury exposure was linked to a delay of the N145 component (Murata et al., 1999a,b). Interestingly, they also observed that the N75 and P100 tended to be positively associated (i.e. delayed) with mercury concentrations measured in the mother's hair (indicator of prenatal exposure) but negatively associated with mercury concentrations collected in child hair at the time of testing. In the current study, after controlling for covariates, prenatal mercury exposure as estimated by mercury concentrations in the cord blood was not related to a delay of the N150, but to a significant delay of the P100 at 30% contrast, although simple Pearson correlations also indicated a delay of the N150 at 95% contrast ($r = 0.19$, $p < 0.05$, $n = 77$) and 12% contrast ($r = 0.32$, $p < 0.05$, $n = 65$). Furthermore, we found that blood mercury concentrations collected at the time of testing were strongly associated with shorter latencies of the N75 and P100 components.

The latter result, which is in agreement with the observations of Murata et al. (1999a,b), is somewhat difficult to reconcile with clinical studies that show delays of the VEP latency following a dysfunction of the visual system (Halliday, 1992). One may ask whether exposure to methylmercury reduces brain volume and, consequently, decreases the time it takes retinal input to reach the occipital cortex. In accordance with this hypothesis, it has been shown that acute and heavy exposure to methylmercury can produce anthropometric malformations (Harada, 1995). More recently, Ramirez et al. (2000) have reported a tendency, in newborns exposed to mercury, to have a smaller head circumference. A correlation between blood mercury concentrations and head circumference was therefore run in our sample. Although a negative relationship was found ($r = -0.1$), the correlation between these two variables was not significant. The "smaller-brain" hypothesis therefore appears insufficient to explain the shorter latencies observed in our data, although this interpretation remains equivocal considering that head circumference is an indirect and potentially misleading metric of brain size estimation (e.g. Saint-Amour et al., 2005).

On the other hand, there are studies in animals (Gitter et al., 1988; Lilienthal et al., 1994) and humans (e.g. Lamm and Pratt, 1985; Lille et al., 1988) that have observed a similar shortening of latency as a function of toxicant exposure. Urban et al. have found a significant reduction of VEP latency for the N75, and a tendency for the N150 to be delayed among workers exposed to mercury vapors (Urban et al., 1999). These results, therefore, suggest that VEPs are normally generated within an optimal window of time and both latency alterations (delay *and* shortening) might reflect deficits in visual processing. This interpretation is supported by the current literature regarding the timing and the manner in which VEPs are generated. Indeed, the time it takes visual input to reach the primary visual cortex is much shorter (about 50 ms or less) than the measured latency of pattern-reversal VEPs, and only 15–30 additional ms are needed for visual input to recruit extrastriate and associative cortices (Foxe and Simpson, 2002). Although one may assume that early VEPs (e.g. N75) reflect activity from the retino-thalamic pathway and the primary visual cortex, there is clear evidence that the generation of the N150 – and even of the P100 – component involves further visual cortices. Moreover, synchronization of several thousand neurons is required for the generation of the VEPs at the scalp level. Therefore, scalp-recorded VEPs are considered to be the result of a relatively late computation of excitatory and inhibitory postsynaptic potentials involving complex networks and reverberant loops among several neuronal sources. Such computation might therefore explain why "abnormal" VEP latencies can be expressed as a delay or a shortening. Although a latency delay is commonly observed in clinical investigation and although it can easily be explained in term of neural transmission delays, the mechanism underlying latency shortening remains unknown. A plausible hypothesis is that normal sensory processing is disrupted because of selective damages by metallic toxicants to inhibitory circuits (Rothenberg et al., 2002; Urban et al., 1999), which are essential for the normal operation of visual processing. Animal models of methylmercury poisoning support the notion that the activity of the GABAergic system is decreased in the occipital cortex (O'Kusky and McGeer, 1985, 1989). An alternative explanation that could account for the associations between child blood mercury levels and decreased VEP latency in the present study could be that blood mercury concentration at the time of testing is a good proxy for protein intake in fish-eating populations. The inclusion of hemoglobin levels as a confounding variable at the time of testing was likely to

control for poor nutrition, but the assessment of calorie and protein intake could help to better address this issue. The fact that the strength of the mercury-VEP latency association was increased two-fold when child blood Se concentrations were taken into account indicates the need to assess this antioxidant status in similar studies, and raises the possibility that effects of mercury exposure during childhood may not be detected in the absence of such control.

It has been suggested during the last decade that the neurotoxicity of environmental contaminants might be partially or totally attenuated by some vitamins and nutrients that co-occur with seafood consumption, but this hypothesis, to our knowledge, has never been empirically tested in humans. In order to address this hypothesis, the interaction factors cord PCB 153/DHA and child PCB 153/EPA as well as child mercury/child selenium were included in the multiple regression analysis. None of these interaction variables was significantly associated with VEP latencies and amplitudes. This suggests that the adverse effects of environmental contaminants could be independent of these nutrients.

Statistical testing of interactions, however, requires large sample size in order to maximize statistical power. The relatively small number of children tested in this study constitutes a considerable limitation. Nevertheless, in the absence of child PCB 153/child EPA interaction, child EPA was found to be related to a shorter latency. A deficiency in *n*-3 PUFAs during foetal development and early life, especially in DHA, impairs learning and memory and alters visual function (Innis, 2000). By contrast, previous studies have shown that *n*-3 PUFAs supplementation during the first months of life can enhance visual acuity and neural conduction in the visual pathways in human infants born pre-term and at term (Birch et al., 1992; Hoffman et al., 2004; Innis, 2000; Morale et al., 2005). Our results extend the findings of these clinical trials by suggesting that *n*-3 PUFAs could be beneficial for visual processing well after infancy. On the other hand, child EPA was also found to be associated with a decrease of VEP amplitude. Such unexpected association was, however, found only for one dependent variable (P100-to-N150 amplitude at 12% of contrast). Considering the multiple comparisons involved in the analysis and the putative beneficial impact of EPA clearly observed for latency (Table 5), this result appears thus marginal. The absence of clear associations with amplitude might explain why previous VEP studies have targeted latency as the primary metric to assess the impact of environmental contaminants on brain function (e.g. Murata et al., 1999a,b; Vreugdenhil et al., 2004).

The associations observed between selenium and VEP latencies suggest that high intake of selenium during childhood could have a negative impact on the visual system instead of being beneficial or protective against mercury neurotoxicity. Although such associations with selenium were unexpected, it is known that very high intake of essential elements for brain development may turn out to have adverse effects, as it was recently demonstrated for vitamin E (Miller et al., 2005). Selenium toxicity is documented in adults (Hansen, 2000; Yang and Xia, 1995), but there is a lack of reliable scientific information regarding toxicity thresholds for infants and children. The Food and Nutrition Board of the National Research Council (USA) recommends a ''Tolerable Upper Intake Level'' of selenium of 150 μg/day for children 4 to 8 years old, which correspond to an average blood concentration of 2.76 μmol/L (National Academy of Sciences, 2000). The averaged blood selenium concentration observed in the present study was on average twice that limit, i.e. 5.6 μmol/L. Moreover, close to 20% of the children tested had blood selenium concentrations exceeding the maximum safe level recommended for adults, which is from 8 to 10 μmol/L. It is therefore likely that our VEP protocol was sensitive enough to reveal sub-clinical effects of high selenium intake, but further research is needed to address the issue of the threshold for selenium toxicity in paediatric populations.

PCB 153 concentrations at the time of testing were related to a delayed latency of the P100 and N150 components, but this result was also obtained when HCB replaced PCB in the analysis. Therefore, due to very high intercorrelations between PCB congeners and chlorinated pesticides, the effects of specific compounds could not be discriminated. Because of the long half-life of the most prevalent PCBs and chlorinated pesticides, and because the majority (78.2%) of the children tested were breastfed for a long period of time (mean breast-feeding duration among breastfed = 17.4 months), VEP alterations associated with child blood levels must be understood as a result of a bioaccumulative exposure to PCBs and chlorinated pesticides throughout ontogenesis. The present study somewhat corroborates what was found in the Faroe Islands cohort where cord PCB concentrations were not related to VEP components (Grandjean et al., 2001a). However, our results suggest, for the fist time in epidemiological studies, that bioaccumulative effects of pre- and postnatal PCB exposures have subtle effect on the integrity of the visual system. This finding brings new elements in the understanding of brain development alterations induced by PCB exposure. In their study of children highly exposed to PCBs, Chen and Hsu (1994) concluded that prenatal exposure to PCBs affects high-order cortical function, namely the auditory P300, rather than the sensory pathway. Our data, however, challenges this notion by showing that components occurring before the latency range of the P300 can also be affected by PCB exposure.

Although PCB concentrations in Nunavik is about two times lower than those found in the fish-eating populations of the Faroe Islands and Greenland (Muckle et al., 2001b), prenatal exposure to PCB in our Inuit cohort remains three to four times higher than that observed in general populations in southern Québec and United States (see Longnecker et al., 2003 for a comprehensive comparison of PCB levels across several studies). The PCB exposure in the present study is similar to those found in cord and maternal plasma samples of the Rotterdam and Lake Michigan cohorts in which alterations of cognitive and motor functions have been reported (Jacobson and Jacobson, 1996; Vreugdenhil et al., 2002). Overall, these findings, in addition to the significant

associations between VEP responses and child blood PCB concentrations found in the present study, suggest that exposure levels to PCBs in the range of those found in Nunavik (see Table 3) might be sufficiently important to alter brain processing.

With an arithmetic mean of about 120 nmol/L (Table 3), cord blood mercury concentrations measured among the Inuit children is in the order of 10 to 20 times higher than that observed in general population samples in Canada and the United States (Muckle et al., 2001b; Rhainds et al., 1999). When compared with previous cohort studies designed to investigate neurobehavioral effects of prenatal exposure to mercury, the prenatal mercury exposure observed in Nunavik is quite similar to the one observed in the Faroe Islands (Grandjean et al., 1999), slightly lower than the one reported in the Seychelles Islands (Myers et al., 1995b) and substantially lower than in the New Zealand study (Kjellstrom et al., 1986). Except for the Seychelles, these cohort studies indicate that prenatal exposure to mercury might affect the development of cognitive and sensory functions, as shown in our study with VEPs.

Although the clinical significance of the results observed in the present study is difficult to assess, our findings might bring new insights to the understanding of developmental neurotoxicity. Several studies have shown cognitive impairments in children in association with methylmercury and PCB toxicity (Crump et al., 1998; Darvill et al., 2000; Gladen et al., 1988; Grandjean et al., 1997, 1999; Jacobson et al., 1990; Jacobson and Jacobson, 1996, 2003; Patandin et al., 1999; Rogan and Gladen, 1991), but little has been done to assess the functional integrity of sensory processing. This is paradoxical since sensory processing precedes, and therefore impacts, cognitive functioning. For example, the lower performance of infants prenatally exposed to PCBs on the Fagan Test of Infant Intelligence (Darvill et al., 2000; Jacobson et al., 1985), which measure visual information processing and memory, might not indicate only impairment of cognitive abilities, but might also involve some visual sensory deficit. Further studies are needed to assess the relative contribution of low- and high-level information processing to cognitive impairments in children exposed to environmental contaminants.

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